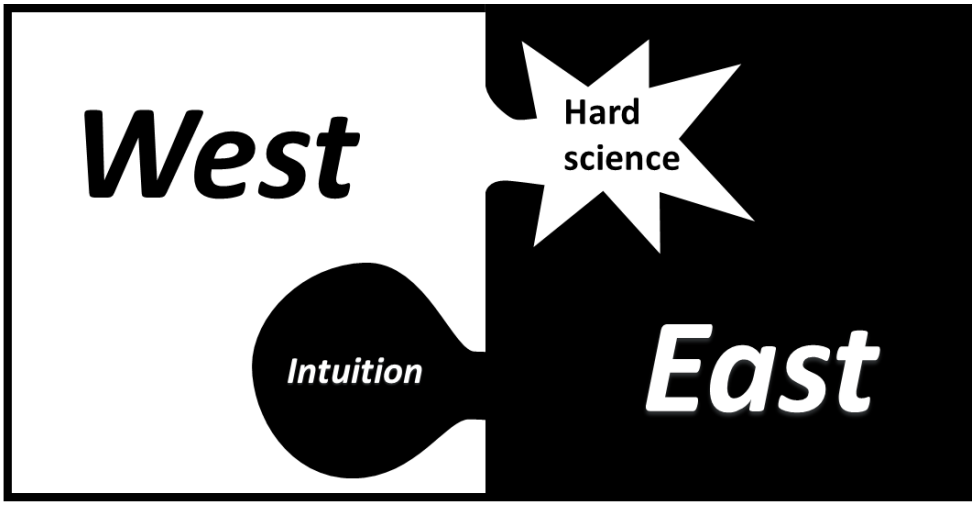
Connecting West and East

Chapter 6

General discussion and summary



The central theme of the thesis is how to make the connection between Western and Eastern medicine. In order to do so, we should start to look at their similarities. **Chapter 1** reviews the similarity between Western medicine and Eastern medicine from an energy perspective. Although Western and Eastern medicine have developed and are applied in different cultures, which explains why their therapeutic concepts are different, we found striking similarities between them. In both worlds, opposing forces provide the energy that flows through networks in an organism, which fuels life. The ability to maintain the balance between these opposing forces, i.e. homeostasis (West) and harmony (East) gives "health" and creates resilience. Moreover, strategies used to treat diseases are strikingly alike. Redox modulating compounds and TCM are added to increase or strengthen connections in the networks to finally adjust the flow of energy and regain harmony. The energy perspective provides a basis to integrate Eastern and Western medicine and results in the researches described in other chapters of the thesis.

In TCM, herbs are combined in the proper ratio to increase therapeutic effects and to reduce side effects. However, when TCM is used in the West, often only the herb with the most active ingredients is used. By the incorrect use, TCM is likely to give side effects. This is exemplified in **Chapter 2** by the cardiovascular side effects of Ma Huang observed in the West. We found that the contractile response on placental arteries by Ma Huang in isolation is much higher than that of Ma Huang in combination with the other herbs in Ma Huang Decotion (MHD) as it used in TCM. This indicates that the interplay of the various compounds in MHD mitigate the side effects of Ma Huang. Previous studies showed that the other herbs in MHD also increase the therapeutic efficacy of Ma Huang. This confirmed that cardiovascular side effects reported in the West resulted from the improper use of Ma Huang in isolation, also without the supervision of knowledgeable professionals. To fully value its benefit, TCM should be used properly as developed over centuries in China.

Especially, combination of drugs might causes serious side effects in the West. For example, many Western drugs show some affinity for the acetylcholine receptors in the brain. When these drugs are combined, this may give substantial blocking of these receptors, which is known as anticholinergic accumulation (AA). Some bioactives in Traditional Chinese Medicine (TCM) are known to block acetylcholine receptors and thus potentially cause AA. In **chapter 3**, the AA of TCM was screened by quantifying the displacement of [^{3}H] pirenzepine on acetylcholine receptors in a rat brain homogenate. We used a new unit to express AA, namely the Total Atropine Equivalents (TOAT). It was found that the TOAT of various herbs used in TCM was very diverse but relatively low and even negative for some herbs. This is indicative for the broadness of the pallet of ingredients used in TCM. Three TCM formulas were screened for AA: Ma Huang Decotion (MHD), Antiasthma Simplified Herbal Medicine intervention (ASHMI), and Yu Ping Feng San (YPFS). The TOAT of ASHMI was indicative for an additive effect of herbs used in it. Nevertheless, it can be calculated that one dose of ASHMI is probably too low to cause AA. The TOAT of YPFS was practically zero. This points to a protective interaction of AA. Remarkably, MHD gave a negative TOAT, indicating that the binding to the acetylcholine receptors was increased, which also circumvents AA. In conclusion, our results indicate that TCM is not prone to give AA and support that there is an intricate interaction between the various bioactives in TCM to cure diseases with minimal side effects.

Redox modulation compounds or flavonoids are potent reactive oxygen species (ROS) scavenger and strengthen the defense system against oxidative stress in the body. Two of the most studied flavonoids, (-)-epicatechin and quercetin, were studied to further unravel their redox modulation effects in the network.

When scavenging ROS, (-)-epicatechin will donate two electrons and become (-)-epicatechin quinone, and thus take over part of the oxidative potential of the ROS. In **chapter 4**, the chemical reactivity of (-)-epicatechin quinone was determined. When the reactivity would be spread out over the entire molecule, i.e. over the AC-ring and B-ring, this would lead to partial epimerization of (-)-epicatechin quinone to (-)-catechin quinone. In our experiments, (-)-epicatechin quinone was generated with tyrosinase. The formation of (-)-epicatechin quinone was confirmed by trapping with GSH, and identification of (-)-epicatechin-GSH adducts. Moreover, (-)-epicatechin quinone could be detected using Q-TOF/MS despite its short half-life. To detect the epimerization, the ability of ascorbate to reduce the unstable flavonoid quinones into the corresponding stable flavonoids was used. The results showed that the reduction of the formed (-)-epicatechin quinone by ascorbate did not result in the formation of an appreciable amount of (-)-catechin. Therefore it can be concluded that the chemical reactivity of (-)-epicatechin quinone mainly resides in its B-ring. This could be corroborated by quantum chemical calculations. Understanding the stabilization of the (-)-epicatechin quinone will help to differentiate between flavonoids and to select the appropriate compound for a specific disorder.

In **chapter 5**, the redox modulating effects of quercetin (Q) was studied. Q has very strong antioxidant activity because of the combination of two pharmacophores, AC-ring and B-ring. In Q, the C2 position has the highest occupied molecular orbital because of the interaction of the 3OH group with 5 and 7OH groups, as well as the 4 carbonyl group. Therefore, Q will donate an electron to a free radical from the C2 positon. The formed quercetin radical (Q$^{\bullet}$) is stabilized by intra molecular scavenging between C2 and C1' and the delocalization of the unpaired electron in the B-ring, in which 4'OH group plays an essential role. After intra molecular scavenging, the AC-ring in Q$^{\bullet}$ is restored to donate another electron to a free radical from the C2 position. This leads to the formation of the two-electron oxidation product, quercetin quinone methide (QQ). QQ is stabilized through tautomerization. Of the four tautomers of QQ, the tautomer with the carbonyl groups at the maximum distance from each other, is the most abundant one. In line with the hard-soft-acid-base concept, the soft electrophile QQ preferably reacts with soft nucleophiles such as thiols over hard nucleophiles such as ascorbate and water. QQ can be recycled to Q by ascorbate, and glutathione can adduct to the 6 or 8 position of its AC-ring to form a glutathione adduct. It is speculated that the adduction of thiol group of KEAP1 by QQ will adapt cells against oxidative stress by increasing the synthesis of endogenous antioxidants. However, direct evidence for the adduction of KEAP1 by QQ has not been proved. Our systematic review paves the path to compare the redox modulating activity of Q to that of other polyphenols on a molecular level. This forms the fundament to ultimately select the appropriate redox modulating compound for a specific redox mediated disorder to increase our health.